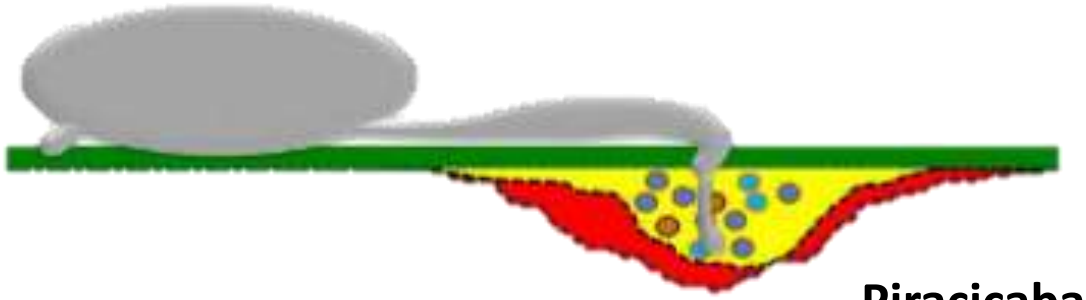
Universidade de São Paulo
Escola Superior de Agricultura Luiz de Queiroz

Differential accumulation of callose, arabinoxylan and cellulose in nonpenetrated versus penetrated papillae on leaves of barley infected with *Blumeria graminis* f. sp. *hordei*

Jamil Chowdhury[1], Marilyn Henderson[1], Patrick Schweizer[2], Rachel A. Burton[1], Geoffrey B. Fincher[1] and Alan Little[1]

[1]ARC Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia; [2]Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany

Arnaldo Esquivel Fariña



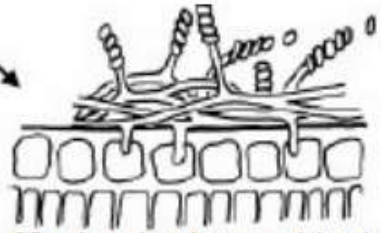
Piracicaba - 2016

Barley powdery mildew
Blumeria graminis f. sp. hordei

É uma das principais doenças que afectam monocotiledóneas. Como todas as espécies dos oídios Erysiphales, é um agente biotrofico obrigatório (Ambos e Spanu, 2004).

Blumeria graminis f. sp. hordei

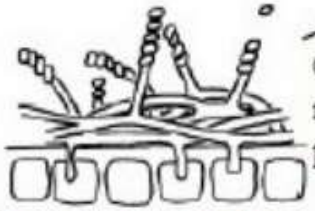
Fungal survival in the debris as it awaits favourable conditions for development



Hyphae develop resulting in conidia production

Guided by wind, spores are blown away and land on a host plant

New fungal infection on wheat developed from conidiospores

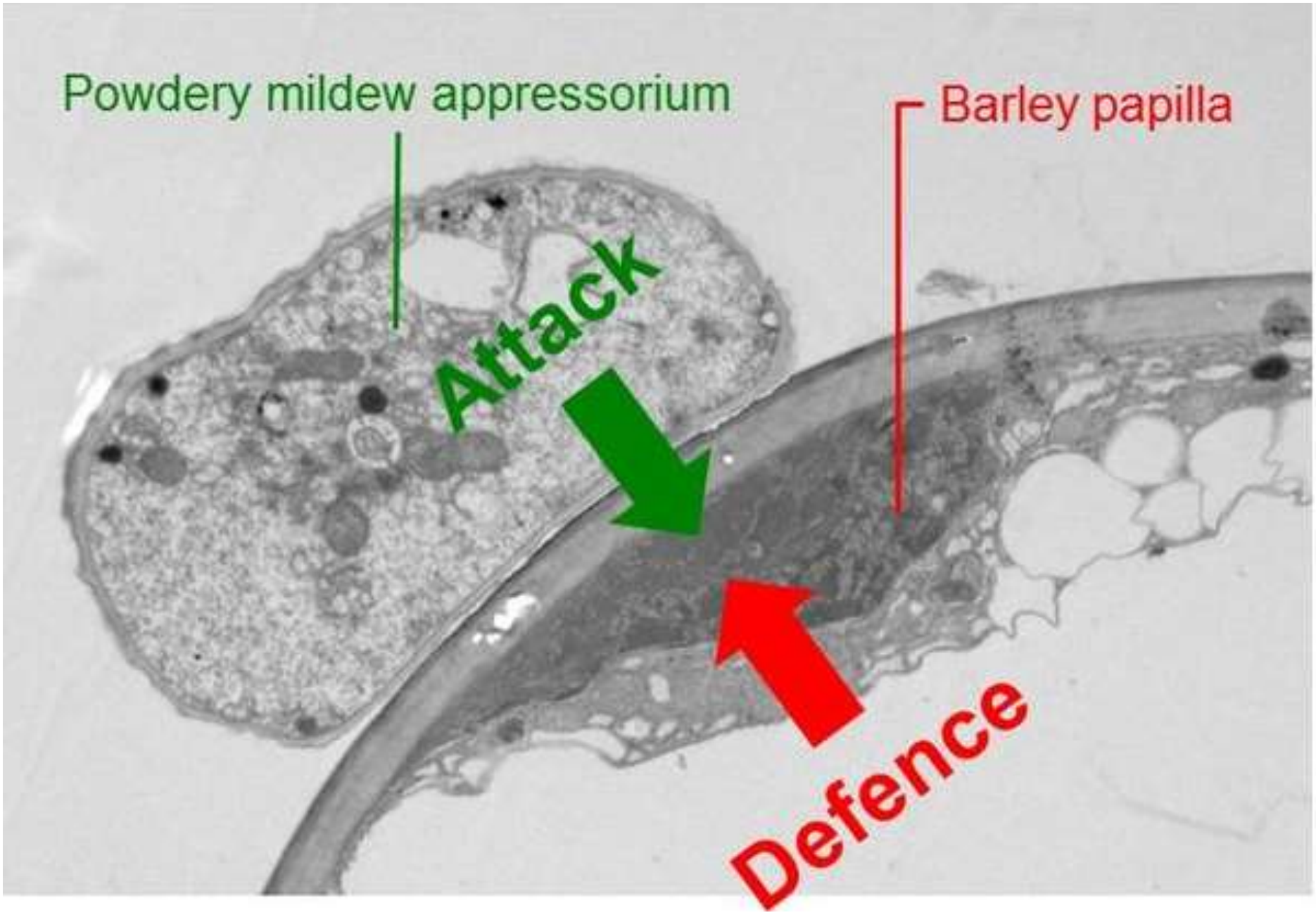


Conidia resulting in new infection in the production period

The resultant mycelia produce new conidiospores

Chasmothecia (fruit body) may be formed on the wheat tissue

Conidia developing on wheat tissue


Powdery mildew appressorium
Barley papilla
Attack
Defence

papillae can be classified as:

- **effective**
- **ineffective**

In general, most studies have focused on correlating papilla effectiveness with the:

1. timing of the formation of papillae
2. their architecture and their size
3. **papilla composition.**

Barley (*Hordeum vulgare*) papillae have been shown to contain:

1. **Callose**
2. Phenolics (lignin and phenolic conjugates)
3. Arabinogalactan proteins
4. Antimicrobial components
5. Inorganic elements
6. Reactive oxygen species (ROS)

(Aist, 1976; Zeyen et al., 2002; Underwood, 2012).

callose-rich ineffective papillae found (Aist, 1976)

Callose gene: **GLUCAN SYNTHASE-LIKE 5 (GSL5)**

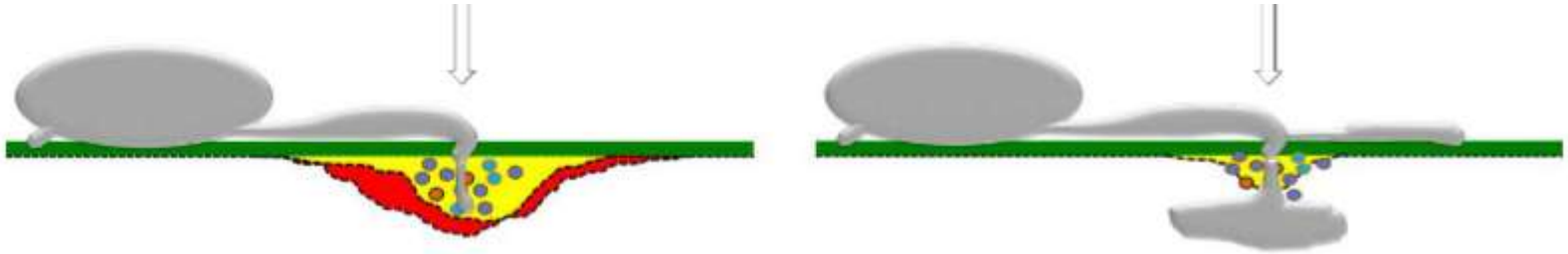
Mutantes deficiente: pouca diferencia entre tratamentos Arabidiopsis – *Golovinomyces cichoracearum*

Overexpresed: confere resistencia a *Golovinomyces cichoracearum*

more information to improve understanding of the structural and compositional basis of papilla effectiveness?

Objetivo

Comparar a composição de papilas eficientes e não eficientes, na mesma variedade de cevada, utilizando sondas específicas para diversos polissacarídeos.



Materiais e metodos

Inoculation was carried out on the adaxial leaf surface of 10-d-old detached leaves pinned on agarose gel in petri dishes. Conidia were inoculated at an average density of 100 mm2.

Immunofluorescence labelling

Immunogold labelling

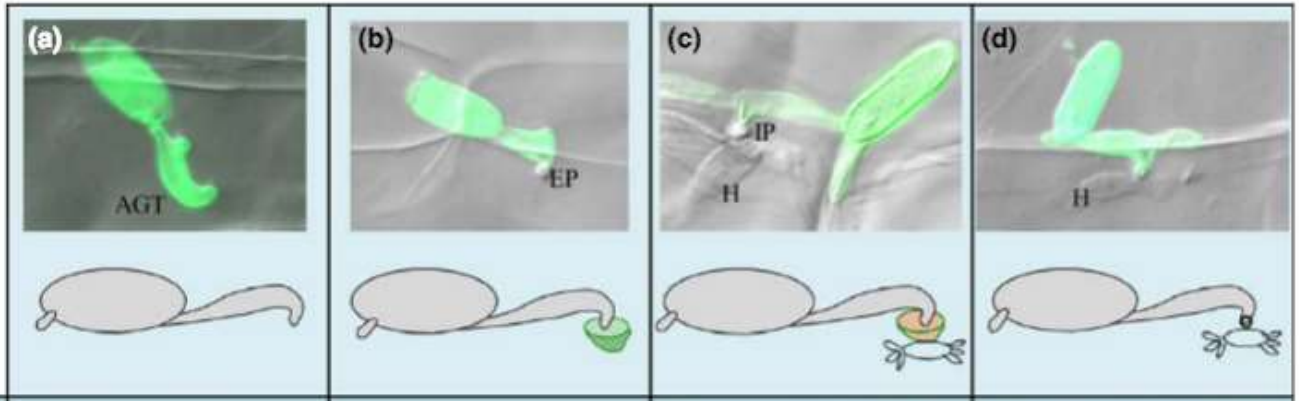
For light and electron microscopy

scanning confocal microscope

- Fungal structures were stained with **wheat germ agglutinin (WGA)** conjugated with Alexa Fluor 488.
- Cellulose was labelled with a 0.01% solution of Pontamine Fast Scarlet 4B

Probe	Target antigen
BG1	(1,3;1,4)-β-glucan
BGM C6	(1,4)-β-Mannan; galactomannan
CBM3a	Crystalline cellulose
LAMP2H12H7	(1,3)-β-glucan
LM5	(1,4)-β-galactan
LM6	(1,5)-α-arabinan
LM8	Xylogalacturonan
LM9	Feruloylated galactan
LM10	(1,4)-β-Xylan
LM11	(1,4)-β-Xylan, arabinoxylan
LM15	Xyloglucan
LM19	De-esterified homogalacturonan
LM20	Methylesterfied homogalacturonan

Importance of effective papillae in pre-invasion resistance in the barley–Bgh interaction



Bgh structure		Appressorium	Appressorium	Appressorium & haustorium	Appressorium & haustorium
Papilla		None	Effective	Ineffective	None
Susceptible line % (Golden Promise)	24 h	12.5	76.5	4.9	6.1
	48 h	6.9	79.4	6.2	7.5
	72 h	4.4	74.5	12.3	8.8

Fig. 1 Possible infection outcomes from barley–*Blumeria graminis* f. sp. *hordei* interactions after the formation of an appressorium germ tube (AGT). Overlay images show *B. graminis* f. sp. *hordei* conidia labelled with chitin-specific WGA-AF 488 (green) and underlying papillae and haustoria visible using the reflective index of differential interference contrast (DIC) microscopy. (a) Unsuccessful penetration without any visible host responses, (b) unsuccessful penetration due to the formation of an effective host papilla, (c) successful penetration through an ineffective host papilla, and (d) successful penetration in the absence of a detectable papilla. Percent frequencies were estimated from 200 conidial observations at each time-point using the susceptible barley cultivar 'Golden Promise'. EP, effective papilla; IP, ineffective papilla; H, haustorium.

Presence of wall polysaccharides in effective barley papillae

Table 1 Relative signal intensity of cell wall-specific probes in the barley papillae and halo regions formed against *Blumeria graminis* f. sp. *hordei*

		Papillae		Halo region		Uninfected epidermal wall	
Probe	Target antigen	IF	IGE-LM	IF	IGE-LM	IF	IGE-LM
BG1	(1,3;1,4)-β-glucan	NS	NS	NS	NS	+++	+++
BGM C6	(1,4)-β-Mannan; galactomannan	NS	+	NS	NS	+	+
CBM3a	Crystalline cellulose	+++	+++	+	+	+++	+++
LAMP2H12H7	(1,3)-β-glucan	++	++	+	+	NS	NS
LM5	(1,4)-β-galactan	NS	NS	NS	NS	NS	NS
LM6	(1,5)-α-arabinan	NS	NS	NS	NS	NS	NS
LM8	Xylogalacturonan	NS	NS	NS	NS	NS	NS
LM9	Feruloylated galactan	NS	NS	NS	NS	NS	NS
LM10	(1,4)-β-Xylan	NS	NS	NS	NS	NS	NS
LM11	(1,4)-β-Xylan, arabinoxylan	+++	+++	++	++	+	+
LM15	Xyloglucan	+	NS	NS	NS	++	+
LM19	De-esterified homogalacturonan	+	+	NS	NS	+	+
LM20	Methylesterfied homogalacturonan	+	NS	+	NS	+	NS

It is important to note that the avidity of each probe is different and therefore only qualitative comparisons can be made between tissues that have been treated with the same probe. All intensities were observed in 1-µm-thick transverse sections using the susceptible barley cultivar 'Golden Promise'. NS, no signal detected; +, limited labelling; ++, moderate labelling; +++, strong labelling; IF, immunofluorescence; IGE-LM, immunogold enhancement in light microscopy.

crystalline cellulose

1. callose

2. arabinoxylan

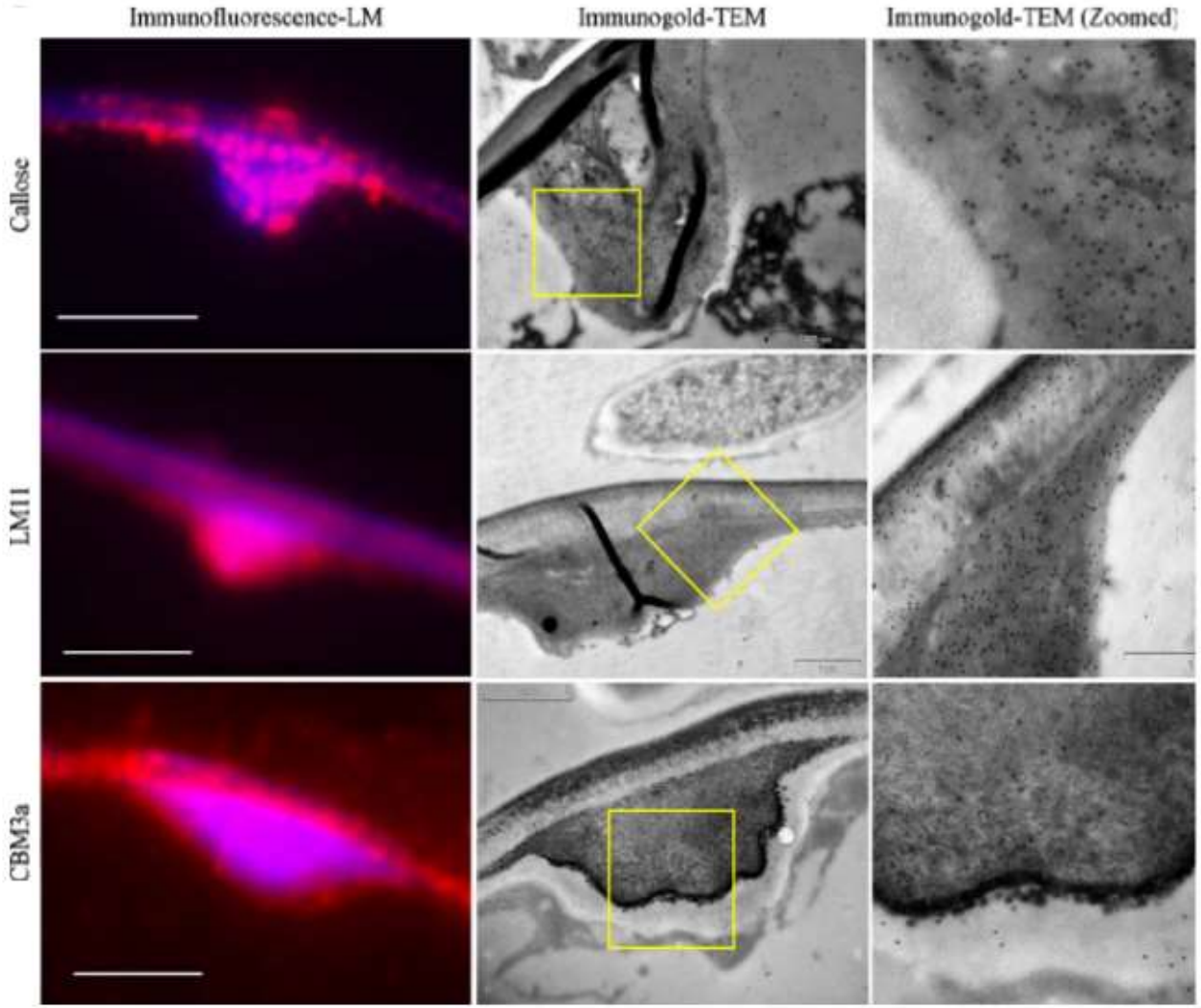


Fig. 2 Transverse sections of barley papillae formed against *Blumeria graminis* f. sp. *hordei* at 24 h after inoculation (hai) labelled with callose-, arabinoxylan- and crystalline cellulose-specific probes. All intensities were observed in 1-µm-thick transverse sections using the susceptible barley cultivar 'Golden Promise'. Left column, red fluorescence, AF555-secondary antibodies attached to respective primary antibodies; blue fluorescence, phenolic acid-associated autofluorescence; bars, 5 µm. Middle column, immunogold-labelled transmission electron micrograph; bars, 1 µm. Right column, magnified view of boxed area from second column. LM, light microscopy; TEM, transmission electron microscopy.

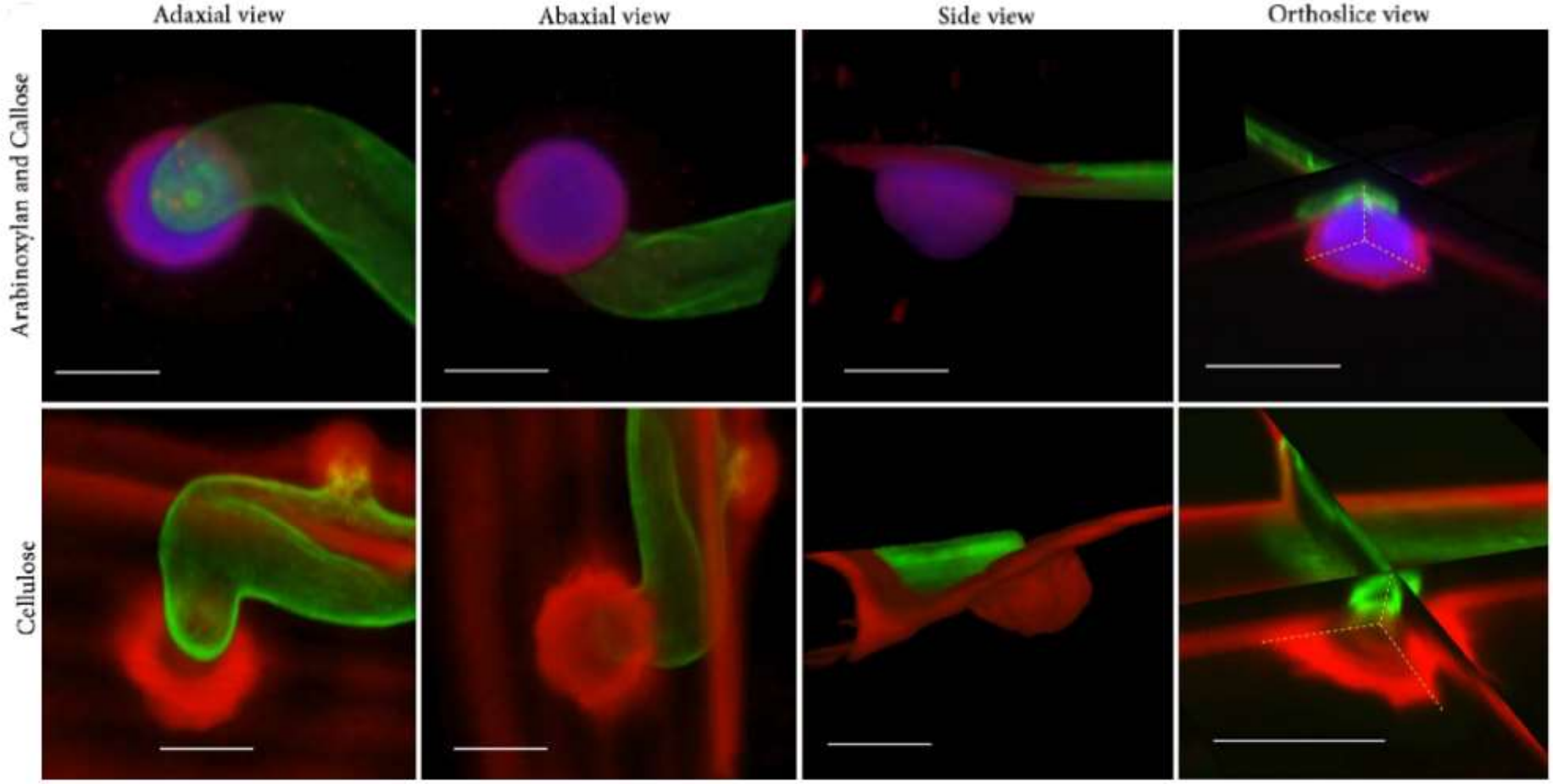


Fig. 3 Confocal microscopy of polysaccharide deposition patterns in effective barley papillae formed against *Blumeria graminis* f. sp. *hordei* at 24 h after inoculation (hai). All intensities were observed using the susceptible barley cultivar 'Golden Promise'. Fungal appressoria were labelled with WGA-AF488 (green). Upper panels, different views of a papilla labelled with an arabinoxylan probe (LM11; red) and a callose probe (aniline blue; blue). Lower panels, different views of a papilla labelled with a cellulose probe (Pontamine fast scarlet 4B; red). Bars, 5 μm.

The effective papillae are therefore composed of two layers; the innermost layer containing callose and arabinoxylan

the second outer layer containing cellulose and arabinoxylan.

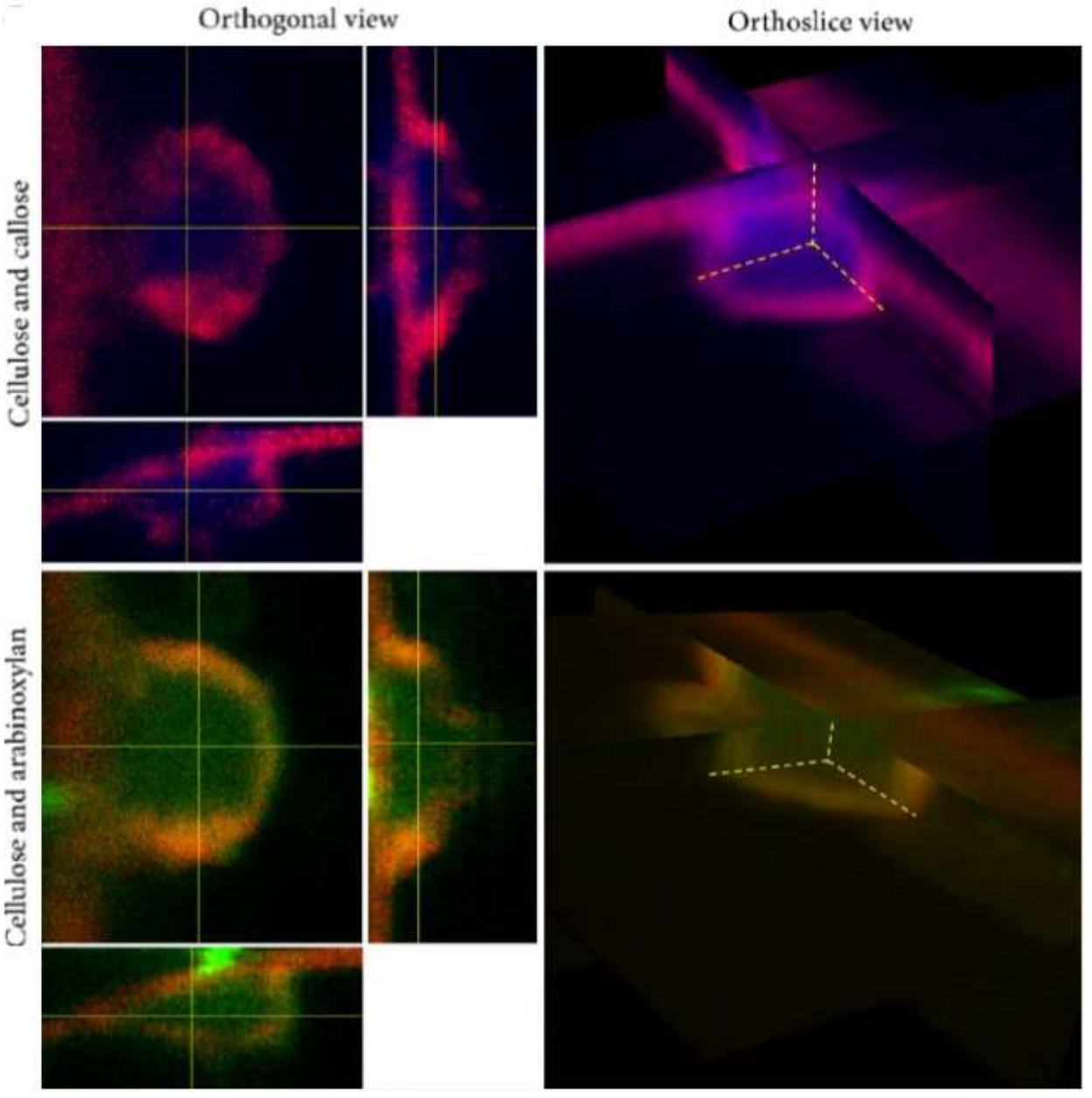


Fig. 4 Confocal microscopy of polysaccharide deposition patterns in effective barley papillae formed against *Blumeria graminis* f. sp. *hordei* at 24 h after inoculation (hai). All intensities were observed using the susceptible barley cultivar 'Golden Promise'. Orthogonal views of virtual sections show papilla labelling from top and side angles. Papilla were labelled with the same probes as Fig. 3, but with different colours: callose (blue), cellulose (red) and arabinoxylan (green).

Association of polysaccharide-specific probes with effective and ineffective barley papillae

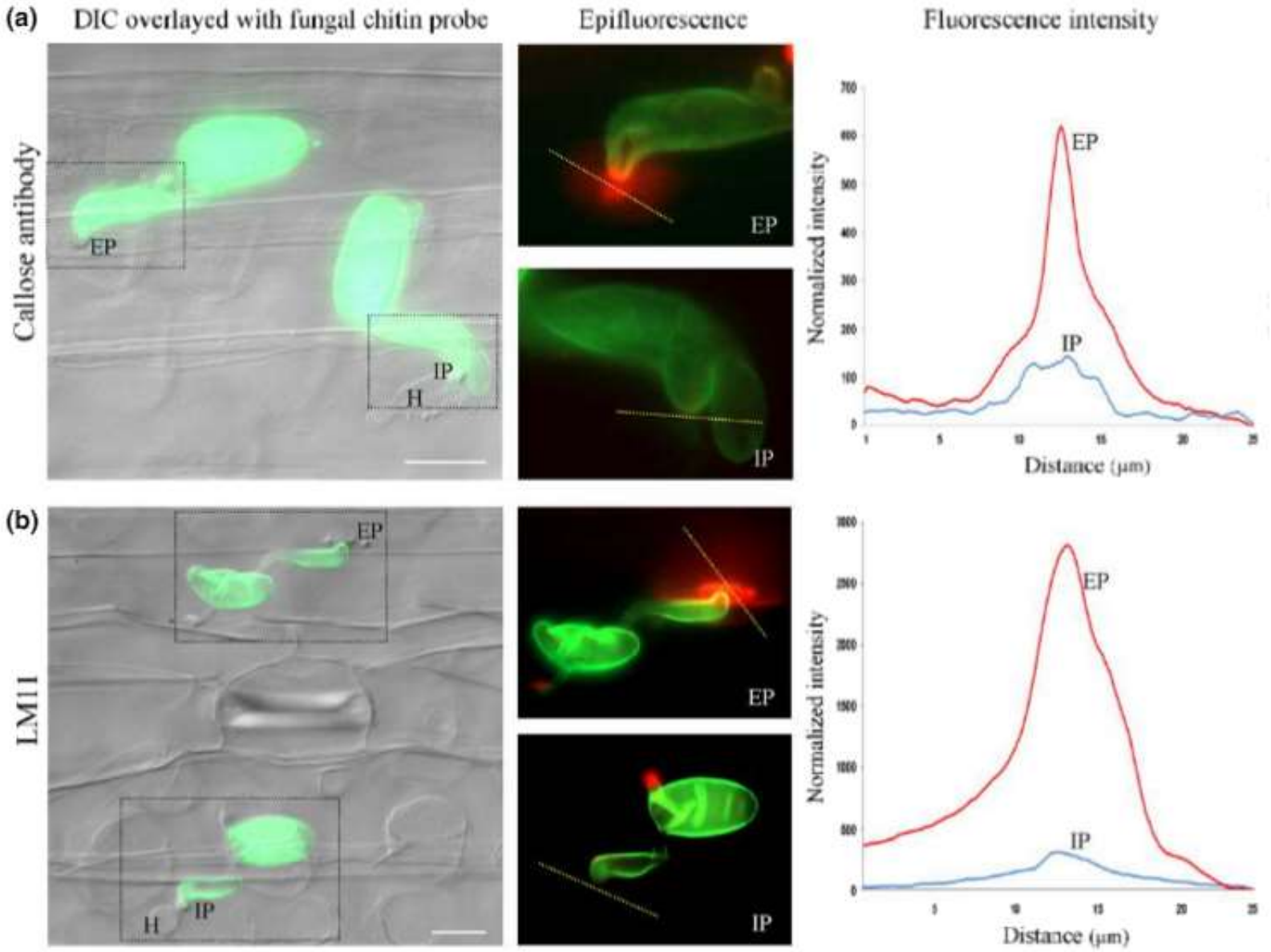
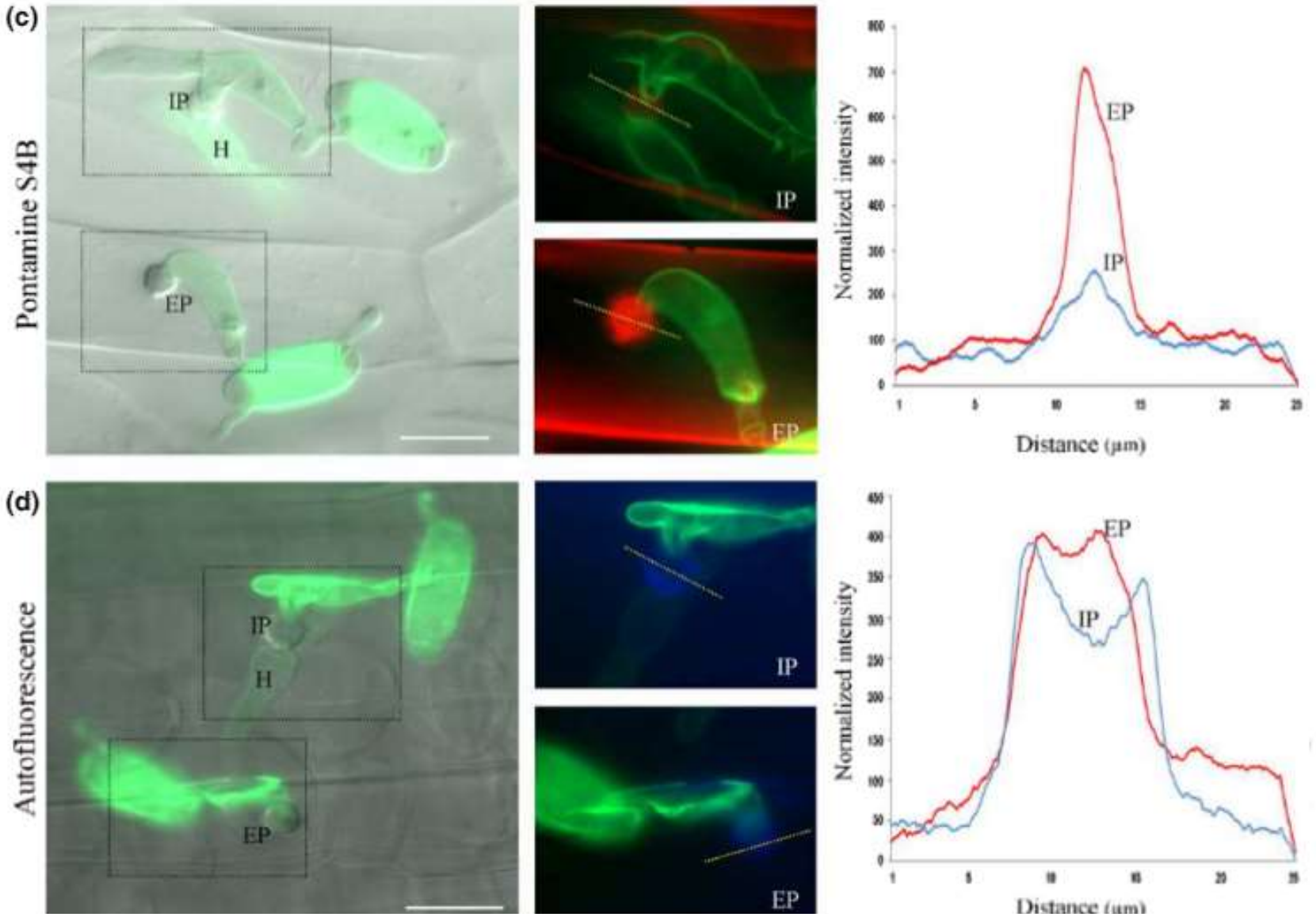


Fig. 5 Labelling of callose-, arabinoxylan- and cellulose-specific probes and phenolic acid-associated autofluorescence in effective and ineffective barley papillae formed against *Blumeria graminis* f. sp. *hordei* at 24 h after inoculation (hai). All intensities were observed using the susceptible barley cultivar 'Golden Promise'. (a–d) Fungal appressoria labelled with WGA-AF488 (green). (a–c) Polysaccharide-specific probes (red) and (d) autofluorescence (blue). Fluorescence intensity profiles correspond to yellow dashed lines showing the distribution of observed labelling in effective and ineffective papillae. EP, effective papilla; IP, ineffective papilla; H, haustorium. Bars, 20 µm.



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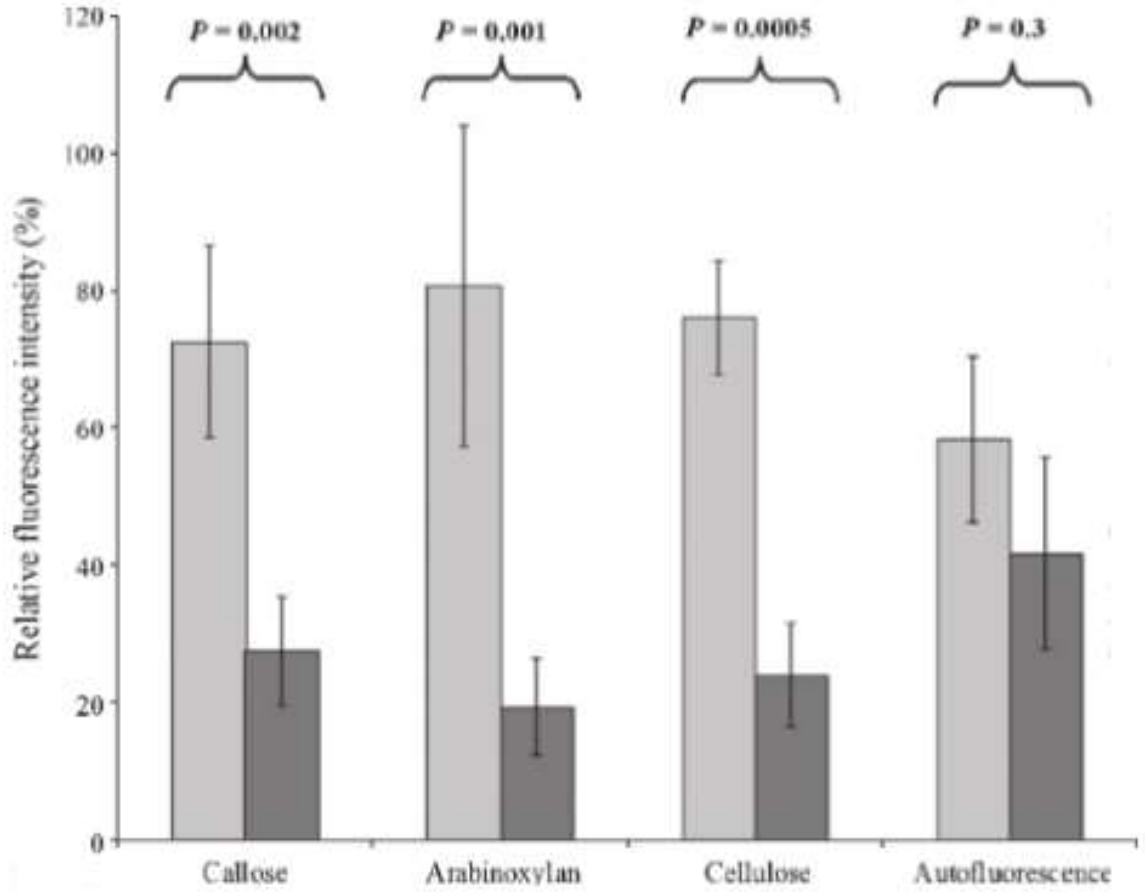

Fig. 6 Relative fluorescence intensity in effective (light grey bars) and ineffective (dark grey bars) barley epidermal papillae at 24 h after inoculation (hai). All intensities were observed using the susceptible barley cultivar 'Golden Promise'. For each probe, the average maximum intensity was calculated using the maximum intensity found in the fluorescence intensity profile of fifty papillae and normalized against background tissue staining. In order to directly compare the different probes in one graph, the relative fluorescence intensity was calculated as a percentage of the highest maximum found for each probe. Error bars, ± SD of the mean. *P* values of Student's *t*-test are indicated above each set.

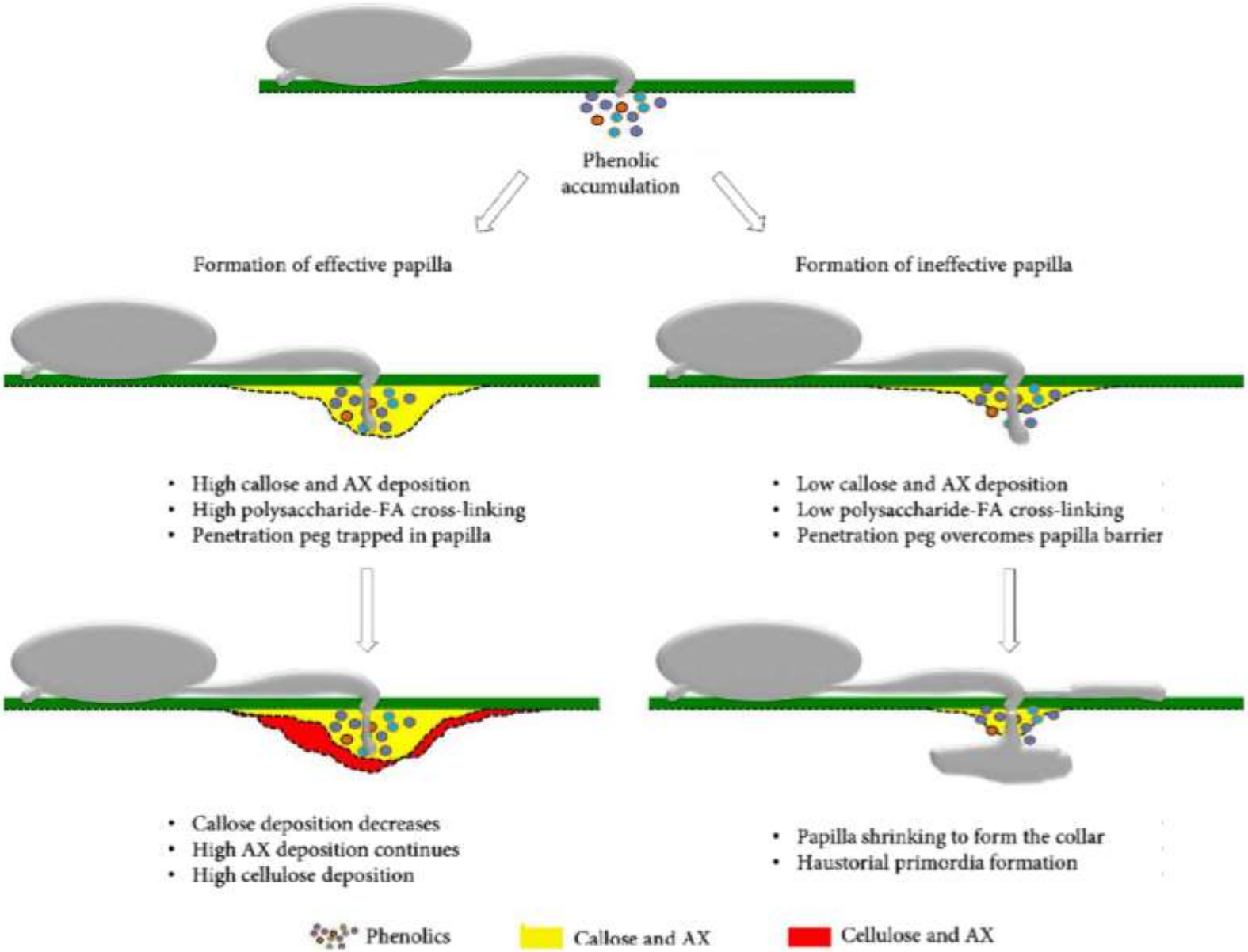

Fig. 7 Putative model depicting deposition of polysaccharides and phenolics in effective and ineffective papillae during the barley–*Blumeria graminis* f. sp. *hordei* interaction. AX, arabinoxylan; FA, ferulic acid.

Obrigado